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Antibiotic resistance characterization of food borne pathogens from India

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Abstract
Food borne diseases are increasing globally and disease-causing pathogens are becoming resistant to antibiotics. The health hazards of such antibiotic resistant pathogens are life threatening and also puts economic burden on the patient. This study determined antibiotic resistance of *E. coli*, *Salmonella*, *Staphylococcus* isolated from roadside food vendors from Lucknow and Pune, India. A total of 374 food samples were collected for this study. The food samples were analysed in laboratory by conventional biochemical techniques and prepared quantitative media. Confirmed isolates were further subjected to antimicrobial susceptibility testing by the Agar disc diffusion technique. Results showed that 77 *Salmonella* isolates showed resistance to more than two antibiotics while 239 *Staphylococcus* showed resistance to more than two antibiotics. 159 *E. coli* isolates showed multidrug resistance. The multiple antimicrobial resistance exhibited by the *Salmonella*, *Staphylococcus* and *E. coli* in this study is an indication of possible multidrug resistant strains present in road side food items. Therefore, roadside food vendors as well as common people should be made more aware regarding food borne diseases caused due to multidrug resistant pathogens.

Keywords: *Salmonella*, *Staphylococcus*, *E. coli*, antibiotic resistance, food borne diseases

Introduction

Food is essential for humans to survive and quality of food matters as it directly affects human health. Thus, it is necessary to consume nutritious and healthy food. Human beings have been consuming raw food from the ancient era. The raw food was cleaned with water to reduce the microbial load. As the civilisation progressed, humans started processing raw food and preparing different types of processed food. In this process, to minimise the microbial load, after washing, salt, sugar, spices, condiments, heating was used. As these processes gave taste to the food, they were favoured over raw food. Fruits, vegetables, meat, eggs, milk were processed and various new processed foods were consumed. But, as the popularity of these products increased, quality while preparing the processed food was compromised and focus on reducing the microbial load was neglected.

This gave rise to food poisoning by pathogenic microorganisms like *E. coli*, *Staphylococcus*, *Salmonella*, *Shigella*, Yeast and Mould etc. Food poisoning gives rise to clinical symptoms like abdominal pain, nausea, vomiting, diarrhoea, dysentery, fever, chills, gastroenteritis, pyrexia and sometime life-threatening septic shock (Helrich, 1990). As these food poisoning cases along with death toll was rising, antibiotic Penicillin was discovered by Alexander Fleming in 1928. Antibiotics are natural compounds produced by microorganisms which kills other microorganisms, are used to treat bacterial infections worldwide. But, when the antibiotics were used widely, pathogens evolved mechanism for inactivating the drug and resistant strains have been developed (Davies and Davies, 2010) [8]. As new food pathogens were discovered, many modifications were done in the structure of the antibiotic and synthetic antibiotics were formulated (Davies and Davies, 2010) [8]. As the nature selects survival of the fittest, pathogenic microorganisms evolved themselves against these new emerging antibiotics and developed resistance mechanisms to survive. This is known as antibiotic resistance and has been a major problem in modern era (Nair *et al*. 2018, Holzel *et al*. 2018) [15, 12].

Food borne infections are most lethal as quality of the food consumed may be compromised when prepared on large scale. Roadside food items are known to cause food poisoning as cleanliness, cooking, and hygiene parameters are not properly followed. These food vendors are not aware of the facts of food poisoning and its health hazards. As the food is produced on bulk; vegetables, fruits, meat and poultry products are many times supplemented with antibiotics (Qamar *et al*. 2020; Abatcha *et al*., 2020; Anukampa *et al*., 2017; Upadhyaya *et al*.,2017; Rasheed *et al*. 2014; Singh *et al*., 2007; Madhulika *et al*., 2004). [16, 1, 3, 21, 17, 18, 14]. Livestock, fruits, and vegetables may also be contaminated by food handlers, farmers, and animal caretakers who carry multi-drug resistant bacteria (Britto *et al*., 2019; Iyer *et al*., 2019; Amare *et al*., 2019; Bantawa *et al*, 2019; Doyle, 2015; Arathy *et al*, 2011; Threlfall, 2002; Teuber 1999) [7, 13, 2, 5, 9, 4, 19, 20].

Antibiotic resistance is rising major concern as it affects human health directly. When these patients are subjected to antibiotic treatment, they failed to respond to the treatment

as the pathogens are already antibiotic resistant. Thus, treatment becomes complicated, expensive and increases risk to patient. Hospital stay increases with financial burden while quality of life decreases due to heavy medications affecting the innate immunity. Patients may become immunocompromised and thus may get the infections easily after the treatment. To prevent such incidences possible and effective approaches such as using farming practice, natural antibiotics, nano-antibiotics, lactic acid bacteria, bacteriocin, cyclopeptide, bacteriophage, synthetic biology and predatory bacteria as alternatives for traditional antibiotics have been suggested (Hashempour-Baltork *et al*, 2019) [10].
Food chain in world is huge and especially in India, number of people consuming street food is enormous. Daily cases of food poisoning are increasing and thus there needs to be preventive measures to minimise such cases. It is necessary to educate roadside street food vendors about food poisoning and related health hazards. It is strongly recommended to reduce the microbial load while preparing the food and use organic raw materials. Thus, it was thought worthwhile to study antibiotic resistance shown by food pathogens isolated from roadside food items. Street food vendors from metropolitan cities namely Lucknow and Pune were selected and further microbial analysis of the food samples was carried out.

Materials and Methods

1. Study Area

Two metropolitan cities Lucknow and Pune were selected for the study.

1.1 Chemicals and Media

Standard media and laboratory chemicals were procured from Himedia Pvt. Ltd. and were used in laboratory for microbiological analysis. Agar powder Bacteriological grade (HiMediaGRM026) was used for solidification of media. Selective ready prepared media MacConkey broth (HiMediaM539) for *E. coli*, *Salmonella Shigella* agar (HiMediaM108) for *Salmonella* and *Staphylococcus* agar no. 110 (HiMediaM521) for *Staphylococcus* were used.

2. Sampling

A total of 374 samples were obtained for the study. Food samples were purchased from markets in the study area from food vendors just the way they were sold to any customer but obtained in separate labelled sterile polythene bags and immediately transported to the Cytogene laboratory (Lucknow) and Hydrotech laboratory (Pune) for bacteriological analyses.50 food samples were collected by Cytogene laboratory in Lucknow city in July and August 2020 and 324 food samples by Hydrotech laboratory in Pune city from December to February 2021. The samples were stored at 4°C at the laboratory until processing.

3. Laboratory Procedures

3.1 Isolation of pathogenic bacteria from Lucknow food samples

All food samples were collected, and 1 grams of each food sample were weighed aseptically. 1 g of food sample was placed in 9 ml (10%) sterile saline, homogenized using a sterile spatula (dilution 10^{-1}). From this 10^{-1} dilution, serial dilutions up to 10^{-9} were done. Loopful of 10^{-9} dilution was spread on nutrient agar medium. Microorganisms isolated on nutrient agar medium were streaked on selective media such as MacConkey agar for identification of *E. coli*, Mannitol Salt Agar (MSA) for *S. aureus* and Xylose Lysine Deoxycholate agar (XLD) for *Salmonella*. The plates were kept at 37 ℃ for 72 hours. Colonies observed on these selective media were used for further analysis.

3.2 Conventional Biochemical Tests

The following conventional morphological and biochemical tests were carried out on the presumptive *Salmonella* and *E. coli* isolates: colony characteristics, morphological and biochemical tests which include Indole test, Methyl red test, Voges Proskauer test, Citrate test, catalase test, oxidase test were performed according to the standard protocols.

3.3 Isolation of pathogenic bacteria from food sample from Pune

All food samples were collected, and 1 grams of each food sample were weighed aseptically. 1 g of food sample was placed in 9 ml (10%) sterile saline, homogenized using a sterile spatula (dilution 10^{-1}). From this 10^{-1} dilution, serial dilutions up to 10^{-5} were done. 1 ml from 10^{-5} dilution was spread on different ready prepared media plates. All the plates were incubated at 37 ℃ for 72 h. After incubation, all colonies were enumerated.

3.4 Antimicrobial Susceptibility Testing

For all the isolates, In vitro susceptibility of the *Salmonella*, *E. coli* and *Staphylococcus* isolates to 7 antimicrobial agents, was investigated according to the Kirby-Bauer disk diffusion susceptibility test protocol (Bauer *et al*., 1996) [6]. The antibiotics tested were Ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), cefoxitin (30 µg), erythromycin (15 µg), streptomycin (10 µg), tetracycline (30 µg).
Inoculum was prepared by selecting five different colonies of 1mmsize approximately, from 24 hours old culture grown on Agar plates and incubated at 35 ± 2°C. Colonies are suspended in 5ml of sterile 0.85% Saline to yield1 x 10^6- 5 x 10^6cells /ml. This culture was added to Muller Hinton Agar presterilized media. The culture suspension was added at 40°C and media was poured. Plates were allowed to solidify. Discs were applied using aseptic technique and the discs with centres at least 24 mm apart were deposited. The plates were inverted and placed in an incubator set to 35 ± 2°C within 15minutes after the discs are applied. The plates were examined after 20-24 hours of incubation. The resulting zones of inhibition were uniformly circular and there was a semi-confluent lawn of growth. Reading were taken at 48 hours only when insufficient growth was observed after 24 hours incubation.

4. Statistical Analysis

The data recorded during the course of investigation was statistically analysed using SPSS software and conclusion was drawn accordingly.

Results

1. Study Area

The study was carried out in Lucknow and Pune in India.

2. Sampling

Total of 374 food samples from roadside food vendors from Lucknow and Pune were collected. These food items which included samosa, wada pav, idli, batata wada, udid wada, kanda bhaji, batata bhaji, bhel, masala puri, ragada puri, ragada pattice, shev puri, dosa, uttapa, kacchi dabeli, shev pav, pohe, sev usal, kachori, sandwich, tea, upma, bread

pattice, medu wada, sabudana wada, sabudana khichadi, lassi, dhapata, appe, thalipeeth, boiled egg, egg bhurji, egg omlet, egg rice, egg pattice, gol bhaji, coffee, sheera, sambar, chatni, misal, paneer veg roll, moong bhaji, rice, flower mattur sabji, dal, french peas sabji, patti samosa, mirchi bhaji, farsan, dal wada. The samples were stored at 4℃ at the laboratory until processing.

3. Laboratory Procedures

3.1 Isolation of pathogenic bacteria from Lucknow food samples

Total of 135 isolates were obtained from 50 food samples collected from different food vendors from Lucknow.

3.2 Conventional Biochemical Tests

Biochemical tests were done to confirm detection of food borne pathogen. The results were compared with laboratory standards and confirmation of *E. coli*, *Salmonella* and *Staphylococcus* was done.

3.3 Isolation of pathogenic bacteria from food sample from Pune

From 324 food samples 492 isolates were obtained including *E. coli*, *Salmonella* and *Staphylococcus.* Himedia selective media were used and isolated colonies on plates were compared to respective Himedia data sheets for identification.

3.4 Antimicrobial Susceptibility Testing

All the isolates were subjected to antibiotic susceptibility test and response to the antibiotics was recorded as susceptible, intermediate and resistant. Zone of inhibition in cm was calculated and compared with standard table given for every antibiotic with specific concentration in Himedia antimicrobial susceptibility systems data sheets. The results were compared with CLSI standard.

Table 1: Antibiotic susceptibility test of Lucknow food sample isolates

Sample code	GEN (10µg)	S (10µg)	E (15µg)	CX (30µg)	TE (30µg)	CIP (5µg)	CH (30µg)
AZI001A/1	0 (R)	10 (R)	0 (R)	0 (R)	9 (R)	11 (R)	12 (R)
AZI001A/2	0 (R)	0 (R)	8 (R)	0 (R)	10 (R)	0 (R)	15 (I)
AZI001A/3	0 (R)	5 (R)	8 (R)	5 (R)	5(R)	0(R)	0 (R)
AZI002A/1	11 (R)	0 (R)	13 (R)	0 (R)	15 (I)	5 (R)	9 (R)
AZI002A/2	5 (R)	6 (R)	0 (R)	4 (R)	5 (R)	0 (R)	9 (R)
AZI003A/1	0(R)	16 (S)	0(R)	14 (R)	13 (R)	17 (I)	18 (S)
AZI003A/2	0(R)	0(R)	0(R)	12 (R)	13 (R)	11 (R)	10 (R)
AZI003A/3	0 (R)	0 (R)	12 (R)	13 (R)	12 (R)	0 (R)	9 (R)
AZI004A/1	0 (R)	0(R)	9(R)	9(R)	0(R)	7(R)	9(R)
AZI004A/2	0(R)	9(R)	8(R)	9(R)	0(R)	9(R)	10(R)
AZI004A/3	0(R)	0(R)	9(R)	0(R)	9(R)	9(R)	10(R)
AZI005A/1	0(R)	0(R)	0(R)	0(R)	0(R)	4(R)	4(R)
AZI005A/2	0(R)	13 (I)	12(R)	9(R)	10(R)	12(R)	0(R)
AZI005A/3	10(R)	12 (I)	11(R)	0(R)	7(R)	10(R)	12(R)
AZI006A/1	9(R)	11(R)	10(R)	10(R)	9(R)	10(R)	11(R)
AZI006A/2	0(R)	5(R)	0(R)	0(R)	0(R)	0(R)	5(R) (R)
AZI006A/3	0(R)	12 (I)	12(R)	13(R)	12(R)	12(R)	12(R)
AZI006A/4	0(R)	14 (I)	13(R)	0(R)	12(R)	12(R)	12(R)
AZI007A/1	4(R)	0(R)	0(R)	0(R)	0(R)	0(R)	4(R)
AZI007A/2	0(R)	11(R)	14(I)	10(R)	10(R)	10(R)	10(R)
AZI007A/3	4(R)	12(I)	13(R)	3(R)	10(R)	0(R)	10(R)
AZI007A/4	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	3(R)
AZI008A/1	0(R)	10(R)	10(R)	10(R)	0(R)	0(R)	13(I)
AZI008A/2	7(R)	0(R)	0(R)	0 (R)	0(R)	0(R)	5(R)
AZI009A/1	0(R)	9(R)	9(R)	8(R)	8(R)	8(R)	11(R)
AZI009A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI010A/1	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI010A/2	0(R)	8(R)	8(R)	8(R)	0(R)	9(R)	9(R)
AZI010A/3	0(R)	0(R)	12(R)	14(R)	14(R)	0(R)	15(I)
AZI011A/1	0(R)	0(R)	0(R)	3(R)	0(R)	0(R)	0(R)
AZI011A/2	0(R)	9(R)	11(R)	8(R)	9(R)	10(R)	12(R)
AZI012A/1	0(R)	14(I)	12(R)	14(R)	0(R)	0(R)	0(R)
AZI012A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI012A/3	0(R)	12(I)	11(R)	10(R)	10(R)	0(R)	14(I)
AZI012A/4	0(R)	10(R)	7(R)	0(R)	0(R)	0(R)	9(R)
AZI013A/1	0(R)	9(R)	9(R)	8(R)	0(R)	9(R)	0(R)
AZI013A/2	13(I)	0(R)	11(R)	12(R)	0(R)	13(R)	15(I)
AZI013A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI014A/1	9(R)	0(R)	0(R)	9(R)	8(R)	9(R)	10(R)
AZI014A/2	6(R)	12(I)	0(R)	0(R)	9(R)	0(R)	0(R)
AZI014A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI015A/1	3(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI015A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI015A/3	0(R)	10(R)	8(R)	10(R)	10(R)	9(R)	9(R)
AZI016A/1	0(R)	8(R)	8(R)	0(R)	10(R)	8(R)	10(R)
AZI016A/2	0(R)	4(R)	4(R)	7(R)	0(R)	0(R)	7(R)

AZI016A/3	0(R)	0(R)	0(R)	7(R)	0(R)	0(R)	0(R)
AZI016A/4	0(R)	0(R)	0(R)	0(R)	8(R)	0(R)	0(R)
AZI017A/1	17 (S)	0(R)	19 (S)	8(R)	0(R)	18(I)	20(S)
AZI017A/2	0(R)	0(R)	0(R)	0(R)	4(R)	4(R)	11(R)
AZI017A/3	0(R)	11(R)	11(R)	0(R)	0(R)	9(R)	12(R)
AZI018A/1	0(R)	10(R)	0(R)	12(R)	13(R)	12(R)	12(R)
AZI018A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI018A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI018A/4	14(I)	14(I)	14(I)	13(R)	14(R)	14(R)	18(S)
AZI019A/1	0(R)	11(R)	13(R)	14(R)	14(R)	11(R)	14(I)
AZI019A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	4(R)
AZI019A/3	0(R)	4(R)	8(R)	0(R)	0(R)	0(R)	8(R)
AZI019A/4	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI020A/1	0(R)	14(I)	0(R)	13(R)	14(R)	14(R)	14(I)
AZI020A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI020A/3	0(R)	0(R)	0(R)	4(R)	0(R)	4(R)	8(R)
AZI021A/1	0(R)	0(R)	5(R)	0(R)	5(R)	0(R)	8(R)
AZI021A/2	0(R)	9(R)	9(R)	14(R)	14(R)	8(R)	0(R)
AZI022A/1	0(R)	15(S)	0(R)	17(R)	17(I)	17(R)	19(S)
AZI022A/2	0(R)	15(S)	0(R)	15(R)	0(R)	0(R)	15(I)
AZI023A/1	0(R)	12(I)	0(R)	14(R)	11(R)	11(R)	14(I)
AZI023A/2	4(R)	4(R)	6(R)	0(R)	0(R)	0(R)	0(R)
AZI024A/1	0(R)	0(R)	12(R)	0(R)	0(R)	12(R)	13(I)
AZI024A/2	15(S)	17(S)	16(I)	0(R)	16(I)	0(R)	19(S)
AZI025A/1	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI025A/2	0(R)	13(I)	13(R)	15(R)	0(R)	14(R)	16(I)
AZI026A/1	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI026A/2	12(R)	0(R)	11(R)	5(R)	10(R)	0(R)	11(R)
AZI026A/3	0(R)	0(R)	13(R)	9(R)	9(R)	11(R)	12(R)
AZI027A/1	8(R)	10(R)	0(R)	9(R)	11(R)	0(R)	14(I)
AZI027A/2	14(I)	15(S)	13(R)	12(R)	13(R)	13(R)	16(I)
AZI027A/3	0(R)	0(R)	13(R)	12(R)	12(R)	0(R)	13(I)
AZI028A/1	0(R)	14(I)	14(I)	0(R)	15(I)	14(R)	17(I)
AZI028A/2	0(R)	6(R)	0(R)	0(R)	4(R)	4(R)	4(R)
AZI029A/1	0(R)	0(R)	0(R)	0(R)	7(R)	0(R)	6(R)
AZI029A/2	0(R)	13(I)	0(R)	13(R)	11(R)	12(R)	13(I)
AZI029A/3	0(R)	11(R)	10(R)	12(R)	11(R)	0(R)	12(R)
AZI031A/1	10(R)	0(R)	0(R)	10(R)	18(I)	0(R)	15(I)
AZI031A/2	0(R)	9(R)	0(R)	0(R)	0(R)	8(R)	0(R)
AZI031A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI031A/4	0(R)	0(R)	10(R)	0(R)	0(R)	0(R)	0(R)
AZI031A/5	10(R)	5(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI032A/1	0(R)	13(I)	12(R)	11(R)	14(R)	14(R)	13(I)
AZI032A/2	0(R)	8(R)	10(R)	0(R)	8(R)	9(R)	10(R)
AZI032A/3	0(R)	15(S)	15(I)	15(I)	10(R)	16(I)	0(R)
AZI033A/1	0(R)	0(R)	13(R)	15(I)	12(R)	10(R)	18(S)
AZI033A/2	0(R)	10(R)	11(R)	12(R)	11(R)	11(R)	12(R)
AZI033A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI033A/4	0(R)	10(R)	11(R)	10(R)	11(R)	0(R)	13(I)
AZI034A/1	10(R)	0(R)	12(R)	13(R)	12(R)	10(R)	0(R)
AZI034A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	7(R)
AZI034A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	8(R)
AZI035A/1	11(R)	11(R)	12(R)	13(R)	11(R)	11(R)	14(I)
AZI035A/2	15(S)	14(I)	12(R)	11(R)	0(R)	0(R)	14(I)
AZI036A/1	0(R)	10(R)	8(R)	8(R)	8(R)	7(R)	12(R)
AZI036A/2	0(R)	0(R)	0(R)	0(R)	0(R)	4(R)	5(R)
AZI037A/1	0(R)	10(R)	13(R)	12(R)	13(R)	0(R)	12(R)
AZI037A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI038A/1	10(R)	12(I)	0(R)	0(R)	11(R)	14(R)	12(R)
AZI039A/1	0(R)	4(R)	4(R)	0(R)	0(R)	0(R)	7(R)
AZI039A/2	12(R)	11(R)	0(R)	13(R)	13(R)	12(R)	13(I)
AZI040A/1	0(R)	11(R)	10(R)	10(R)	0(R)	12(R)	13(I)
AZI040A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI040A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	7(R)
AZI040A/4	10(R)	0(R)	12(R)	0(R)	10(R)	10(R)	13(I)
AZI042A/1	11(R)	12(I)	9(R)	10(R)	10(R)	0(R)	14(I)
AZI042A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
AZI042A/3	12(R)	0(R)	5(R)	7(R)	8(R)	0(R)	13(I)

AZI043A/1	0(R)	0(R)	0(R)	3(R)	4(R)	0(R)	5(R)
AZI043A/2	0(R)	14(I)	17(I)	15(I)	15(I)	15(R)	16(I)
AZI043A/3	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI043A/4	0(R)	0(R)	11(R)	12(R)	13(R)	11(R)	14(I)
AZI044A/1	0(R)	9(R)	11(R)	11(R)	11(R)	12(R)	13(I)
AZI045A/1	9(R)	12(I)	0(R)	11(R)	10(R)	10(R)	14(I)
AZI045A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI046A/1	0(R)	0(R)	11(R)	11(R)	11(R)	11(R)	15(I)
AZI046A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	2(R)
AZI047A/1	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	5(R)
AZI047A/2	11(R)	0(R)	11(R)	13(R)	14(R)	13(R)	14(I)
AZI047A/3	9(R)	9(R)	0(R)	8(R)	8(R)	0(R)	12(R)
AZI048A/1	0(R)	0(R)	12(R)	13(R)	10(R)	10(R)	14(I)
AZI048A/2	11(R)	0(R)	12(R)	11(R)	0(R)	11(R)	0(R)
AZI048A/3	0(R)	10(R)	9(R)	8(R)	7(R)	7(R)	12(R)
AZI049A/1	12(R)	0(R)	0(R)	12(R)	13(R)	11(R)	14(I)
AZI049A/2	0(R)	0(R)	12(R)	10(R)	13(R)	14(R)	10(R)
AZI050A/1	0(R)	0(R)	15(I)	0(R)	13(R)	15(R)	13(I)
AZI050A/2	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	8(R)
AZI050A/3	10(R)	10(R)	0(R)	12(R)	11(R)	13(R)	14(I)

Key: GEN= Gentamicin, S= Streptomycin, E= Erythromycin, CX= Cefoxitin, TH= Tetracycline, CIP= Ciprofloxacin, CH= Chloramphenicol, µg= Microgram, S= Sensitive, I= Intermediate, R= Resistance.

For Lucknow food sample isolates, antibiotic resistance of food borne pathogens was assessed. Total of 134 isolates were obtained from 50 food samples from Lucknow and assay for antibiotic susceptibility was carried out. Of the total isolates, above 90% were found to be resistant to gentamicin, streptomycin, erythromycin, cefoxitin, tetracycline, ciprofloxacin, while 60% resistant to chloramphenicol. Around 5% of the isolates showed intermediate and sensitive response to the antibiotics tested. This data indicates that Lucknow food samples were heavily contaminated with food borne pathogens and very high numbers of these isolates were multi-resistant to antibiotics tested. This is alarming and when compared to the previous data. Upadhyaya *et al.* (2017) [21]. who have studied the microbiological quality of street side foods and its antibiotic susceptibility. They have collected samosa, chole, panipuri, sandwich and momos of street side carts of Lucknow City and *Escherichia coli, Pseudomonas aeruginosa, Klebsiella, Staphylococcus aureus* were isolated from the food samples. Antibiotic sensitivity test for *E. coli* showed resistance to Itranadozole and Rifampicin. The presence of *Salmonella* which is a threat has also encountered and thus this study represents new data to be looked upon and further detailed analysis is necessary. As compared to this study, our study revealed presence of *Escherichia coli, Salmonella* and *Staphylococcus aureus.* Antibiotic study showed resistance to gentamicin, streptomycin, erythromycin, cefoxitin, tetracycline, ciprofloxacin which indicates presence of multi-drug resistant pathogens in Lucknow food samples.

From Pune, total of 324 food samples from different localities were collected. These food items included samosa, wada pav, idli, batata wada, udid wada, kanda bhaji, batata bhaji, bhel, masala puri, ragada puri, ragada pattice, shev puri, dosa, uttapa, kacchi dabeli, shev pav, pohe, sev usal, kachori, sandwich, tea, upma, bread pattice, medu wada, sabudana wada, sabudana khichadi, lassi, dhapata, appe, thalipeeth, boiled egg, egg bhurji, egg omlet, egg rice, egg pattice, gol bhaji, coffee, sheera, sambar, chatni, misal, paneer veg roll, moong bhaji, rice, flower mattur sabji, dal, french peas sabji, patti samosa, mirchi bhaji, farsan, and dal wada. From these 324 food samples from Pune, using serial dilutions method and ready Himedia agar plates, total of 492 bacteria lpathogens were isolated. Of the total isolates, 77 were *Salmonella*, 239 were *Staphylococcus* and 159 were *E. coli*. This study is unique in the sense that there has not been a single study of food borne pathogens from Pune food vendors. This sample size and location variation represents large data and its analysis enlightens about the deadliest food borne pathogens present in variety of road side foods.

All the isolates were tested for 7 antibiotics and results were surprising. Above 80% of the isolates were found to be resistant to gentamicin, streptomycin, erythromycin, cefoxitin, tetracycline, ciprofloxacin, while 70% resistant to chloramphenicol. All of the isolates showed antibiotic resistance to at least one of the antibiotics while more than 50% of the isolates were resistant to more than 3 or more antibiotics. Figs 1 a, b, c, d are representative of the antibiotic resistance shown by food borne pathogens isolated from Pune food samples.

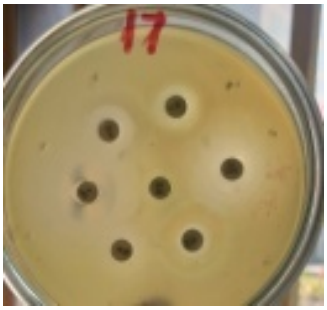

Fig 1a

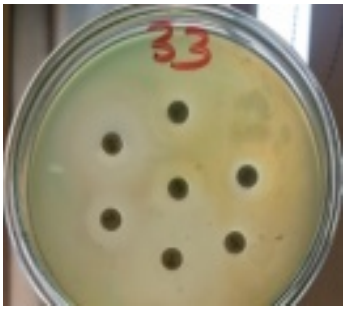

Fig 1b

Fig 1c

Fig 1d

Fig 1a, b, c, d: Multi-drug antibiotic resistance shown by food borne pathogens isolated from Pune food samples

These results indicate that heavy contamination of food borne pathogen in food samples and these pathogens have shown multi-drug resistant. The results are unique and there are no reports as per the author's knowledge till date. Thus, this report becomes a novel study representing huge amount of statistically analysed data giving critical information regarding the antibiotic resistance shown by food borne pathogens in India.

4. Statistical analysis

The data of all the pathogens isolated and its response to 7 antibiotics was statistically analysed using SPSS software and conclusion was drawn accordingly.

Table 2: Antibiotic susceptibility test Results

Place	Result	Gentamicin (10µg)		Streptomycin (10µg)		Erythromycin (15µg)		Cefoxitin (30µg)		Tetracycline (30µg)		Ciprofloxacin (5µg)		Chloramphenicol (30µg)	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%
Lucknow (N=134)	Resistance	128	95.5	108	80.6	126	94.0	131	97.8	128	95.5	131	97.8	92	68.7
	Sensitive	3	2.2	20	14.9	7	5.2	3	2.2	6	4.5	3	2.2	36	26.9
	Intermediate	3	2.2	6	4.5	1	0.7	0	0.0	0	0.0	0	0.0	6	4.5
Pune (N=492)	Resistance	121	24.6	218	44.3	293	59.6	364	74.0	217	44.1	221	44.9	248	50.4
	Sensitive	82	16.7	108	22.0	180	36.6	100	20.3	159	32.3	141	28.7	172	35.0
	Intermediate	289	58.7	166	33.7	19	3.9	28	5.7	116	23.6	130	26.4	72	14.6

In the Table 2, it can be seen that total of 134 samples from Lucknow and 492 from Pune were statistically analysed. Result was interpreted in terms of antibiotic sensitivity, intermediate and resistance response. For Lucknow samples, almost 96% of the isolates were resistant to Gentamicin, 81% to Streptomycin, 94% Erythromycin, 98% Cefoxitin, 96% Tetracycline, 98% Ciprofloxacin and 69% Chloramphenicol. This data clearly indicates that the Lucknow food samples were heavily contaminated with multi-drug resistant pathogens. This also shows that above 70% of the pathogens were resistant to all the antibiotics tested. This is really shocking and can have huge impact on the community health of Lucknow in future. This data needs to be further analysed for what could be the possible causes of this acquired antibiotic resistance and what could be the prevention strategies for the same.

From Pune 324 food samples collected total of 492 food borne pathogens were isolated. All these isolates were subjected to antibiotic sensitivity assay and antibiotic resistance pattern was determined. From the table 1, we can clearly see almost 25% of the isolates were resistant to Gentamicin, 44% to Streptomycin, 60% Erythromycin, 74% Cefoxitin, 44% Tetracycline, 45% Ciprofloxacin and 50% Chloramphenicol. This clearly indicates that out of huge number of samples collected, at least 25% of the isolates are resistance to all the antibiotics. As this data represents most of the prime locations in Pune, the food vendors are selling highly contaminated and multi-antibiotic resistant pathogen containing food. As the sample size and locations are more, further location wise analysis of factors affecting the pathogenicity needs to be determined. This will help in formulating preventive strategies to minimise the food borne outbreak in near future. This study is novel in approach as this is the first study of multi-drug resistant food borne pathogens from Pune.

Discussion

The study clearly indicates multiple antibiotic resistance was found in *E. coli*, *Staphylococcus* and *Salmonella* spp. These results are in agreement with previous reports in India. This study revealed multi-drug resistance of the *Salmonella*, *E. coli* and *Staphylococcus* isolates to commonly used antibiotics, with each isolate resistant to at least four (4) of the antibiotics tested. India has recorded increasing resistance to antimicrobials over the past 25 years. The high (36.6%) susceptibility of the *Salmonella* isolates to erythromycin observed in this study agree with the findings of Anukampa *et al.* (2017) [3]. who reported *Salmonella*completely resistance to Oxacillin (100%) followed by Cefoxitin (30.43%) and Ampicillin (26.10%) in India. The street vended foods of animal origin and associated environment are responsible for transmission of food borne pathogens including *Salmonella*. Britto *et al*. (2019) [7]. have reviewed antimicrobial resistance among *Salmonella typhi* and *Salmonella paratyphi* from patients with enteric fever over two decades in India. The effect of antimicrobial pressure is driving AMR in typhoidal *Salmonella* in India. Iyer *et al*. (2019) [13]. Have reported data about the health-care ecosystem on antimicrobial resistance (AMR) testing and the resistance patterns of typhoidal *Salmonella* isolates in the city of Ahmedabad. Culture and antibiotic sensitivity testing data was collected

from two medical colleges and one corporate laboratory. The data showed concurrent resistance to more than one antibiotic was very high, 88%, among the 67 resistant isolates. It was found that high resistance of typhoidal *Salmonella* isolates to ciprofloxacin in public sector while and azithromycin private sector was may be due to the increased use. Singh *et al*. (2007) [18]. Analysed 974 samples collected from vegetable vendors in two cities, in northern India during the early summer season in 2004. *Salmonella* was isolated from 35 samples while *Escherichia coli* was detected in 181 samples. The majority (82.9%) of *Salmonella* isolates were multidrug resistant. One quarter of the isolates was resistant to ≥10 antibiotics. More than 80% were resistant to sulfamethoxazole, nalidixic acid, and kanamycin. Resistance to imipenem (>20%) and amikacin (>30%) was also common. Also, this study showed resistance of the *E. coli* isolates to at least one of the antibiotics tested. This result is in agreement with the findings by other studies. Rasheed *et al*. (2014) [17]. who reported multidrug resistance among *E. coli* isolates from vegetable salad, raw egg-surface, raw chicken, unpasteurized milk, and raw meat collected from twelve localities of Hyderabad, India. The high resistance (26.9%) observed of the *E. coli* isolates to chloramphenicol in our study is similar with the findings reported by Rasheed *et al*. (2014) [17]. In this study, we have also found out that 14.9% resistance of the Gram-positive *Staphylococcus* was observed against streptomycin in this study. This finding is in agreement with reports by Upadhyaya *et al*. (2017) [21]. They have studied the microbiological quality of street side foods from Lucknow City including samosa, chole, panipuri, sandwich and momos and its antibiotic susceptibility. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella*, *Staphylococcus aureus* were isolated. Antibiotic sensitivity test for *E. coli* showed resistance to Itranadozole and Rifampicin. The multidrug resistance observed in the study is of serious public health concern and requires urgent attention. The present study clearly indicates that preventive measures have to be taken in metropolitan cities to avoid food poisoning outbreak in near future. The report is alarming and serious in the sense that if the food poisoning happens through these food items supplied by food vendors and further as these harmful pathogens are multi-drug resistant, they are not likely to be treatable using broad spectrum antibiotics. This can lead to increase in mortality rate due to food poisoning. Thus, education related to food safety should be made compulsory to food vendors so as to minimise the food poisoning cases through their food items. Further, preventive measures should be taken and quality of the food should be monitored on weekly basis by local government authorities to avoid outbreak.

Conclusion

Antimicrobial resistance (AMR) is a global health and development threat which is among one of the top 10 global public health threats. Due to misuse and overuse of antimicrobials, drug-resistant pathogens evolve. Lack of sanitation and inadequate infection prevention causes the spread of antimicrobial resistant microbes. In addition to cost of AMR, death, disability and prolonged illness results in longer hospital stays. To cure AMR infections, more expensive medicines are required resulting in financial challenges. In this report, 374 food samples from food vendors in and around Lucknow and Pune were analysed. The food samples were analysed for possible microbial pathogenic contamination which can cause food poisoning. It was observed that the food samples were heavily contaminated with pathogenic bacteria which may cause food poisoning. It was also observed that most of the isolated pathogens were resistant to more than 70% of the antibiotic tested. As compared to Indian cases, this report has recent finding which indicates that antimicrobial resistance shown by food borne pathogens is increasing day by day and proper care should be taken to minimise such incidences. It is recommended that health awareness campaigns related to food borne diseases should be implemented in these metropolitan cities. Strict action should be taken by the government authorities on roadside food vendors if the personal health measures are not maintained.

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